IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Petropoulos et al.	Confirmation No.:	2397
Serial No.:	10/077,027	Art Unit:	1648
Filed:	February 15, 2002	Examiner:	U. Winkler
For:	COMPOSITIONS AND METHODS FOR EVALUATING VIRAL RECEPTOR/CO-RECEPTOR USAGE AND INHIBITORS OF VIRUS ENTRY USING RECOMBINANT VIRUS ASSAYS	Attorney Docket No.:	011068-008-999

DECLARATION UNDER 37 C.F.R. § 1.132 OF ROBERT W. DOMS

Commissioner for Patents
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I, Robert W. Doms , hereby state and declare as follows:

1. I am a citizen of the United States of America, residing at 1230 South Leopard Rd, Berwyn, PA, 19312.

2. I received my M.D./Ph.D. degree from Yale University. My Ph.D. research was in Ari Helenius' laboratory and focused on influenza virus entry mechanisms and cell biology. I received postgraduate training in Bernie Moss' laboratory at the NIH where I worked on the cell biology of vaccinia virus and on the biology of the HIV envelope (Env) protein. I also completed a Pathology resisidency at the NIH. In 1992, I became a faculty member of the Department of Pathology and Laboratory Medicine at the University of Pennsylvania School of Medicine where my research focused on the humoral immune response to HIV infection, characterization of the antigenic structure of HIV Env and identification and characterization of HIV coreceptor molecules. My lab was one of the first to identify CCR5 as a major

coreceptor for primary HIV strains. In 2001, I was appointed Chair of the Department of Microbiology at the University of Pennsylvania School of Medicine. Since then, my HIV research has focused on entry mechanisms, virus neutralization and factors that impact susceptibility to entry inhibitors. My lab is also involved in characterizing viruses from patients treated with entry inhibitors to examine resistance mechanisms to entry inhibitors. I am frequently invited to present my research on HIV entry and entry inhibition at national and international conferences.

3. I have carefully reviewed U.S. Application No. 10/077,027 (the "027"application"). The '027 application teaches, *inter alia*, methods for detecting an antibody response capable of blocking infection in a patient infected with a virus. In one set of reactions, a population of nucleic acids encoding Env proteins is obtained from a patient infected with the virus. Cells are co-transfected with an expression vector constructed from the nucleic acids encoding Env proteins and also with viral expression vectors lacking the gene encoding an Env protein but having an indicator gene (e.g. luciferase). The transfected cells will produce so-called pseudotyped virus particles from the Env proteins representing the population. One then harvests these viral particles, contacts them with an antibody preparation from the patient and uses them to infect a second cell line expressing a cell surface receptor (and co-receptor) to which the virus binds in the presence and absence of the antibody preparation, and measures the amount of reporter gene product in the infected cells (e.g. by measuring luciferase activity). In a second set of reactions, cells are transfected with a control *Env* expression vector, which encodes Env proteins with known co-receptor tropism. Reporter gene expression in cells infected with virus containing patient-derived Env is compared to that from the control, in the presence and absence of the antibody preparation. The concentration of preparation required to inhibit reporter gene expression by 50%, for

example, may be determined for patient virus and control, allowing one to detect whether the patient exhibits an antibody response capable of blocking infection.

4. The assay is useful, for example, in detecting an antibody response capable of blocking an infection.

5. The current invention employs a population of virus infecting a patient as opposed to a single clone (or group of clones). The claims recite obtaining a population of *Env* nucleic acids from a patient infected with HIV. The pHIVenv expression vector of the current invention was constructed to accept *Env* sequences amplified from patient plasma samples (*see*, for example, page 47, lines 1-7). Patient-derived sequences inserted into pHIVenv represent the population of virus (*see,* for example, page 48, lines 23-28). To adequately represent a population, many (greater than 100) transformants are used for preparation of pHIVenv (referred to throughout the disclosure collectively as pHIVenv, see disclosure, page 49, lines 18-25). In other places throughout the disclosure, a population of virus is distinguished from clones, single or multiple (see, for example, page 56, lines 30-32; page 57, lines 15-28; page 67, lines 21-23 and page 73, lines 9-13).

6. Before the filing date of the application, detecting an antibody response capable of blocking infection was done by testing resistance of a single clone (or multiple clones). Since a single clone does not represent the complete spectrum of clones within a population of virus infecting a patient, detection of an antibody response using a single clone may not represent the full spectrum of a patient's antibody response to the virus. Furthermore, the clone selection procedure adds time and expense to the assay.

7. My lab is currently characterizing viruses from patients treated with entry inhibitors to examine mechanisms of resistance to these compounds. We amplify the *Env* genes from patient plasma, clone them into an expression vector and select individual clones for analysis. We can successfully characterize these *Env* clones in fusion assays, but have had great

difficulty in generating functional reporter viruses pseudotyped with Env proteins, as in the current invention. We have not taken the approach of trying to generate viral Env proteins from the entire patient population.

8. I am not aware of anyone (before the filing date of the application) who, without the benefit of the '027 application's teachings, has succeeded in making viral particles that express a population of HIV Env proteins from nucleic acids amplified from a patient sample that are useful for detecting an antibody response.

9. I hereby declare that all statements made herein of my own knowledge are true, that all statements made on information and belief are believed to be true, that these statements were made with the knowledge that willful false statements and the like so made are punishable under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

3/12/06
DATE

SIGNATURE OF DECLARANT